Modulations by cAMP and cGMP of the high-voltage activated Ca^{2+} channels in the central neurons of the cotton bollworm, *Helicoverpa armigera*

LI Jie[1,2], DU Yu-Zhe[1], TONG Fan[1], HE Bing-Jun[1], LIU An-Xi[1]

(1. College of Life Sciences, Nankai University, Tianjin 300071, China;
2. Department of Biology, Capital Normal University, Beijing 100037, China)

Abstract: The effects of cAMP and cGMP on the high-voltage activated (HVA) Ca^{2+} channels in the central neurons isolated from the 3rd instar larvae of the cotton bollworm (*Helicoverpa armigera*) were studied using the whole-cell patch clamp technique. With barium as current carrier, the currents of Ca^{2+} channels were recorded by blocking Na^+ and K^+ currents. Extracellular application of 0.1 mmol/L forskolin, a stimulator of adenylyl cyclase (AC), had no effect on the peak amplitude of Ba^{2+} currents. In contrast, 1 mmol/L cGMP in the intracellular solution suppressed the current amplitude significantly and the inhibition was time- and dose-dependent. The results suggest that the activities of HVA Ca^{2+} channels in the cotton bollworm may not be affected by the elevated cAMP, but can be inhibited by the increased cGMP.

Key words: *Helicoverpa armigera*; cyclic AMP; cyclic GMP; high-voltage activated calcium channel; patch clamp

1 INTRODUCTION

The regulations of adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′, 5′-cyclic monophosphate (cGMP) on high-voltage activated (HVA) calcium channels have been extensively investigated in various excitable cells. In cardiac and diaphragmatic cells, the enhancement of current amplitude is modulated by cAMP-dependent phosphorylation of proteins related to calcium channels (Sperelakis and Bournaud, 1991; Fratacci *et al*., 1996). Previous studies suggested that cAMP-dependent phosphorylation is involved in the up-regulation of calcium current in neurons (Gross *et al*., 1990; Liu and Lasater, 1994). Cyclic GMP was also proposed to enhance the slow calcium current in skeletal muscle fibers (Kokate *et al*., 1993). In snail neurons the enhancement of slow calcium current is mediated *via* cGMP-dependent phosphorylation (Paupardin-Tritsch *et al*., 1986). However, the effects of second messengers on calcium channels vary in different preparations. Solntseva and Borisova (1997) discovered that the increase of cAMP suppresses calcium current through neither PKA activation nor the cytoplasmic Ca^{2+} elevation while cGMP induces little enhancement of calcium current in molluscan neurons. Studies have revealed that cAMP has no effect on calcium current in cockroach central neurons (David and Pitman, 1996) and cGMP analogue decreases voltage-sensitive calcium currents in the Aplysia neurons R15 (Levitan and Levitan, 1988). These variations may also be due to the subtypes of channels, tissue specificities and the interactions of intracellular pathways for regulating ion channels.

To date, the effects of intracellular messengers on calcium channels in neurons of the cotton bollworm (*Helicoverpa armigera*) have been barely studied. The present work carried out on its central neurons is the first step to explore the regulations of calcium channels by cAMP and cGMP.

2 MATERIALS AND METHODS

2.1 Isolation of neural cells

The cotton bollworm (*H. armigera*) used in this experiment were initially obtained from cotton fields in Hebei Province and reared indoors at (27 ± 1)℃, with 70% – 80% of relative humidity and 14 h of light.

The thoracic and abdomen ganglia were removed from the 3rd instar larvae, which were anaesthetized with 70% ethanol. The tissues were incubated in insect saline at 4℃ (Hayashi and Levine, 1992; He *et al*., 2002) for 5 min and then were desheathed. The

基金项目：国家自然科学基金项目(30270884, 30100119)
作者简介：李杰，女，1972 年生，博士研究生，主要研究方向为动物神经生理，E-mail: jieli0222@hotmail.com
收稿日期 Received: 2003-10-31; 接受日期 Accepted: 2004-02-12

desheathed ganglia were transferred to solution containing 0.3% trypsin Ⅲ for 10 min at 27℃. After trituration with a fire-polished pipette, cells were plated into plastic culture dishes (diameter of 35 mm) and kept in 1.5 mL culture medium (Hayashi and Levine, 1992) per dish. The cells were allowed to settle and adhere to the surface of the culture dishes for 2 – 4 h at 27℃. All the procedures were carried out under sterile conditions.

2.2 Electrophysiological recording

Neurons with diameter of (19 ± 1) μm were used in this experiment. Micropipettes (1 – 2 μ), made from borosilicated glass capillary tubing, were pulled in a two-step vertical puller (Narishige, PP-830, Japan) and fire polished. The resistance of micropipettes was 2 – 3 MΩ after being filled with intracellular solution. Current recordings were made using whole cell gigaseal method with a PC-IIB amplifier (Huazhong University of Science and Technology) and filtered at 5 kHz. Data acquisition and cell stimulation were performed using IBBC lamp software. Leak currents were subtracted using P/4 procedure. Series resistance was compensated electronically by 80%. All the experiments were carried out at room temperature (20℃ – 25℃).

2.3 Solutions

The intracellular solution contained (mmol/L): CsCl 120, $MgCl_2$ 2, Na_2-ATP 2, EGTA 10, Hepes 10, pH adjusted to 6.6 with 1 mol/L CsOH. The extracellular solution contained (mmol/L): NaCl 100, CsCl 4, $BaCl_2$ 5, $MgCl_2$ 2, Hepes 10, glucose 5, TEA-Cl 20, TTX 0.001, pH adjusted to 6.6 with 1 mol/L NaOH. Forskolin (dissolved in ethanol, stored as stock solution) was added to bath solution and cGMP was dissolved in intracellular solution. CsCl, EGTA and Hepes were purchased from Gibco. CsOH, forskolin and cGMP were purchased from Sigma.

2.4 Data analysis

Statistical comparisons between values from the control and drug-treated cells were analyzed using Student's unpaired *t*-test. Data are expressed as means ± *SE*.

3 RESULTS

3.1 The typical ionic current of calcium channel in the control neuron

Fig.1(A) shows the typical inward Ba^{2+} currents recorded from one central neuron of 3rd instar larvae. The neuron was held at – 70 mV and test voltage depolarized from – 60 mV to + 50 mV for 60 ms with interval of 10 mV. As recorded by He *et al*. (2002), the current was identified as a high-voltage activated Ba^{2+} current, which was activated at about – 40 mV and reached maximal value at about 0 mV. Currents were recorded every five minutes under steady state. Some "run-up" in the current amplitude was exhibited in the beginning and rundown followed with steady decrease in current. By the end of 30 min recording, 74.8% ± 9.8% (n = 9) of the original peak current remained. The time courses to activate Ba^{2+} current of Ca^{2+} channels and to make it reaching maximal value were not affected by the rundown in peak current. Fig. 1(B) shows the current-voltage (I-V) relationships of HVA Ca^{2+} channels (n = 9).

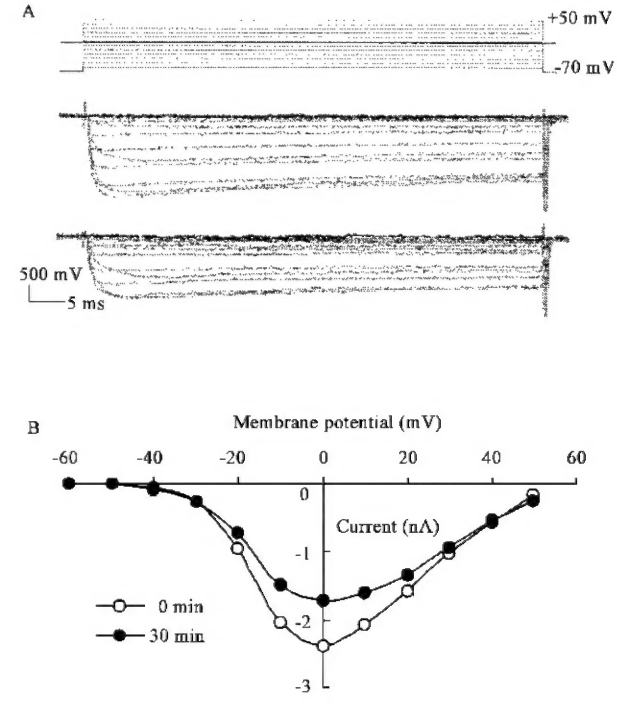

Fig. 1 The typical Ba^{2+} curves and the I-V relationship of Ba^{2+} current *vs* test voltage in the control
A. The upper, middle and lower lines showing the test voltages and the Ba^{2+} currents recorded in one neuron at 0 min and 30 min, respectively; B. I-V relationship of current value *vs* test voltage at 0 min and 30 min.

3.2 The effect of forskolin on calcium channels

Fig.2 illustrates the Ba^{2+} currents recorded in one neuron with the presence of forskolin (0.1 mmol/L) in the bath solution. The currents were recorded 10 min after drug application so that forskolin activated adenylyl cyclase adequately. Although the application of forskolin had brought 0.1% of ethanol to the bath solution, our investigation showed that 0.1% of ethanol had no discernable effect on Ba^{2+} current (data not shown here). The barium currents and the current-voltage relationship were shown in Fig.2 (A and B). The peak current was reduced to 75.2% ± 10.2% (n = 14) of the initial value by the end of 30 min recording. Statistical analysis of peak current values showed that forskolin had little effect on Ba^{2+} current (Fig.2:C). Forskolin, at 0.5 mmol/L, didn't cause

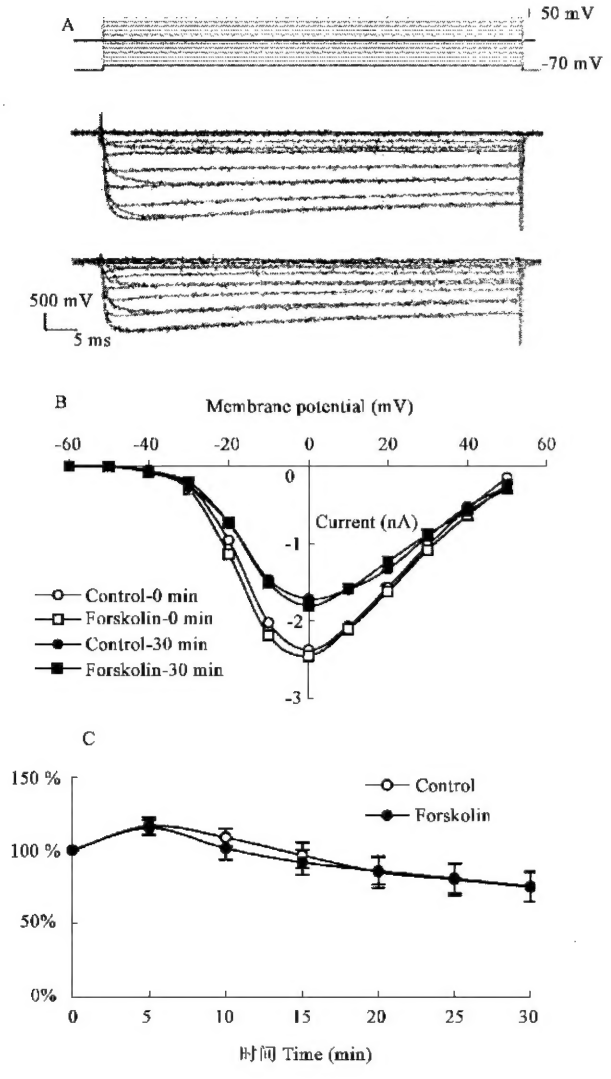

Fig. 2 Forskolin at 0.1 mmol/L has no effect on Ba^{2+} currents
A. The upper, middle and lower lines showing the test voltages and the current curves at 0 min and 30 min, respectively; B. The I-V relationships of current value vs test voltage in the 0.1 mmol/L forskolin-treated and control neurons at 0 min and 30 min, respectively; C. The change rate of peak current amplitude vs recording time in the 0.1mmol/L forskolin-treated and control neurons.

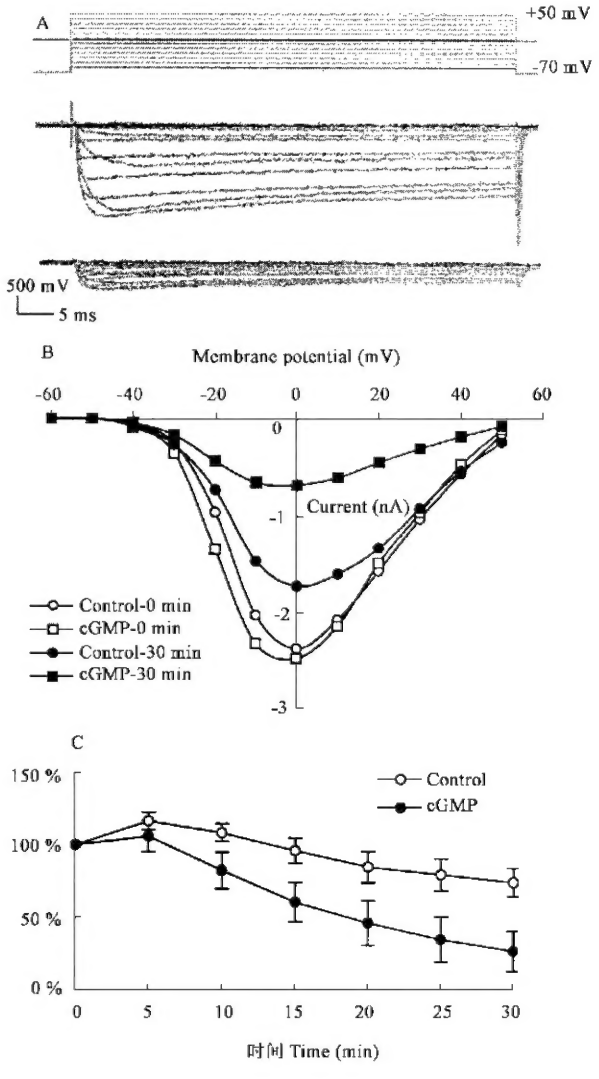

Fig. 3 Cyclic GMP at 1 mmol/L significantly reduced Ba^{2+} currents
A. Ba^{2+} currents induced by test voltages (indicated in the upper lines) at 0 min (the middle lines) and 30 min (the lower lines); B. I-V relationship of current value vs test voltage in the 1 mmol/L cGMP-treated and control neurons at 0 min and 30 min, respectively; C. The change rate of peak current amplitude vs recording time in the 1 mmol/L cGMP-treated and control neurons.

obvious effect on Ba^{2+} current either ($n = 5$, data not shown here). The time courses for Ca^{2+} channels to open and the current to reach maximal amplitude were not different from that of the control.

3.3 The effect of cGMP on calcium channels

Cyclic GMP was dissolved in intracellular solution. After establishing the whole-cell configuration, a period of 5 min was allowed to ensure adequate dialysis of the pipette solution into the cell interior. Ba^{2+} current declined steadily with 1 mmol/L cGMP and the remaining peak currents was 27.3% ± 13.9% (n = 11) of the original value at the end of 30 min recording (Fig.3). In addition, the inhibition of cGMP on Ba^{2+} current was dose-dependent. At 20 min, 46.4% ± 15.5% (n = 11) and 18.3% ± 14.6% (n = 7) of

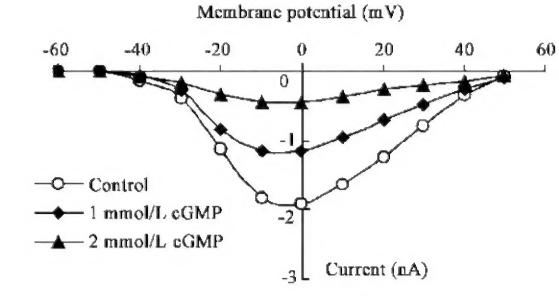

Fig. 4 I-V relationships of current value *vs* test voltage at 20 min in the control, and 1 mmol/L and 2 mmol/L cGMP-treated neurons

peak currents remained for the 1 mmol/L and 2 mmol/L cGMP-treated cells, respectively (Fig.4). At 25 min

of recording, discernable current barely existed in the 2 mmol/L cGMP-treated cells. The time courses to activate Ba^{2+} current of calcium channels and to make it reaching maximal value were not altered by cGMP.

4 DISCUSSION

The present work describes an inhibitory effect of cGMP and zero effect of cAMP on high-voltage activated calcium channels in the cotton bollworm. The question may arise whether 0.1 mmol/L forskolin is enough or not to stimulate adenylyl cyclase. Studies in mouse diaphragmatic cells demonstrated that 0.1 mmol/L forskolin produced 24.4% ± 13.8% increase in L-type Ca^{2+} current magnitude through elevation of cAMP (Fratacci *et al*., 1996). Works in motoneurones of cockroach (David and Pitman, 1996) and in thalamocortical relay neurons of rat (Billa *et al*., 2001) showed that 0.001 mmol/L and 0.02 mmol/L forskolin, respectively, are effective to activate adenylyl cyclase to depress calcium-dependent K^+ current. Based on these excellent works, 0.1 mmol/L forskolin was chosen in this experiment. Besides that, 0.5 mmol/L forskolin was used in some experiments to ensure the adenylyl cyclase to be stimulated completely even if adenylyl cyclase is in scarcity in cotton bollworm neurons. Neither 0.1 mmol/L nor 0.5 mmol/L forskolin can alter the activity of Ca^{2+} channel evidently. How does cAMP interact with Ca^{2+} channels? The generally accepted mechanism by which cAMP exerts its enhancing effect is *via* activation of cAMP-dependent protein kinase (PKA) to phosphorylate proteins related to the channels (Greengard, 1979). In addition, cAMP may also exert its physiological effects by binding to some other proteins that can modulate Ca^{2+} current, for example, G-protein, protein phosphatase, etc. (Palazzolo *et al*., 1989). Besides that, cAMP causes the enhancement of inward current by acting directly with cyclic nucleotide-gated (CNG) cationic channels in some sorts of cells (Kawai and Miyachi, 2001). In this study, the multiple effects of cAMP can not be completely excluded. Furthermore, elevation of cAMP was achieved by applying forskolin, which stimulates adenylyl cylclase to convert ATP to cAMP, instead of adding cAMP directly. Therefore, more works need to be performed to reveal the mechanism of the modulation by cAMP on the Ca^{2+} channels.

Cyclic GMP has been shown to modulate voltage-activated Ca^{2+} channels in various preparations through a cGMP-dependent protein kinase (PKG). Hirooka *et al*. (2000) showed that both cGMP and IBMX, an inhibitor of phosphodiesterase, increase the peak amplitude of N-type Ca^{2+} currents in retinal ganglion cells. Similar enhancement was reported in ventral neurons of the snails (Paupardin-Tritsch *et al*., 1986). In contrast, L-type Ca^{2+} current in rat ventricular myocytes is depressed by cGMP (Sumii and Sperelakis, 1995). This discrepancy may result from the difference between the subunit structures of Ca^{2+} channels in neurons and muscles (Catterall, 1988). The inhibitory effect was also observed in the Aplysia neuron R15 (Levitan and Levitan, 1988). In this study, cGMP exerts significant inhibitory effect on HVA Ca^{2+} currents in neurons of the cotton bollworm. One of the possible pathways through which cGMP depresses the activities of Ca^{2+} channels is referred to the phosphorylation by PKG. The mechanisms underlying different effects of cAMP and cGMP remain obscure. However, the different phosphorylation sites of Ca^{2+} channel may explain the different effects of PKA and PKG. Taking cardiac cells as an example, PKA phosphorylates the α-subunit of Ca^{2+} channels at the Ser1928 residue (Gao *et al*., 1997) whereas PKG phosphorylates at Ser533 (Jiang *et al*., 2000). This difference may exist in insect neurons. The disparity of phosphorylation sites may lead to different effects of cAMP and cGMP in neurons of the cotton bollworm. The work described here is just a beginning to reveal the mechanism underlying the modulations of ionic channels by the intracellular second messengers in insect neurons.

References

Billa G, Meis S, Pape HC, 2001. Modulation of a Ca^{2+}-dependent K^+-current by intracellular cAMP in rat thalamocortical relay neurons. *Thalamus & Related System*, 1: 157 – 167.

Catterall WA, 1988. Structure and function of voltage-sensitive ion channels. *Science*, 242: 50 – 61.

David JA, Pitman RM, 1996. Cyclic-AMP regulation of calcium-dependent K channels in an insect central neuron. *Neurosci. Lett.*, 203: 151 – 154.

Fratacci MD, Shimahara T, Bournaud R, Atlan G, 1996. cAMP-dependent modulation of L-type calcium currents in mouse diaphragmatic cells. *Respiration Physiol.*, 104: 1 – 9.

Gao T, Yatani A, Dell'Acqua ML, Sako H, Green SA, Dascal N, Scott JD, Hosey MM, 1997. cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron*, 19: 185 – 196.

Greengard P, 1979. Cyclic nucleotides, phosphorylated proteins and nervous system. *Fed. Proc.*, 38: 2 208 – 2 218.

Gross RA, Uhler MD, MacDonald R, 1990. The cyclic AMP-dependent protein kinase catalytic subunit selectively enhances calcium currents in rat nodose neurons. *J. Physiol.*, 429: 483 – 496.

Hayashi JH, Levine RB, 1992. Calcium and potassium currents in leg motoneurons during postembryonic development in the hawkmoth *Manduca sexta*. *J. Exp. Biol.*, 171: 15 – 42.

He BJ, Liu AX, Chen JT, Sun JS, Rui CH, Meng XQ, 2002. Acute isolation and culture of nerve cell from the cotton bollworm and the patch clamp study on the voltage-gated ion channels in the cultured neurons. *Acta Entomologica Sinica*, 44(4): 422 – 427. [贺秉军, 刘安西, 陈家童, 孙金生, 芮昌辉, 孟香清, 2002. 棉铃虫幼虫神经细胞的急性分离培养及其电压门控通道的膜片钳研究. 昆虫学报, 44(4): 422 – 427]

Hirooka K, Kourennyi DE, Barnes S, 2000. Calcium channels activation facilitated by nitric oxide in retinal ganglion cells. *J. Neurophysiol.*, 83: 198 – 206.

Jiang LH, Gawler DJ, Hodson N, Milligan CJ, Pearson HA, Porter V, Wray

D, 2000. Regulation of cloned cardiac L-type calcium channels by cGMP-dependent protein kinase. *J. Neurosci.*, 15: 8 214 – 8 222.

Kawai F, Miyachi E, 2001. Modulation by cGMP of the voltage-gated currents in newt olfactory receptor cells. *Neurosci. Res.*, 39: 327 – 337.

Kokate TG, Heiny JA, Sperelakis N, 1993. Stimulation of the slow calcium current in bullfrog skeletal muscle fibers by cAMP and cGMP. *Am. J. Pysiol.*, 265: C47 – 53.

Levitan ES, Levitan IB, 1988. A cyclic GMP analog decreases the currents underlying bursting activity in the Aplysia neuron R15. *J. Neurosci.*, 8: 1 162 – 1 171.

Liu Y, Lasater EM, 1994. Calcium current in turtle retinal ganglion cells. 2. Dopamine regulation via a cyclic AMP-dependent mechanism. *J. Neurophysiol.*, 71: 743 – 752.

Palazzolo M, Katz F, Kennedy TE, Schwartz JH, 1989. Multiple cAMP-binding proteins in Aplysia tissues. *J. Neurobiol.*, 20: 746 – 761.

Paupardin-Tritsch D, Hammond C, Gerschenfeld HM, Naim AC, Greengard P, 1986. cGMP-dependent protein kinase enhances Ca^{2+} current and potentiates the serotonin-induced Ca^{2+} current increase in snail neurons. *Nature*, 323: 812 – 814.

Solntseva E, Borisova O, 1997. Cyclic AMP does not induce the down-regulation of calcium current in molluscan neurons through kinase A activation or cytoplasmic Ca^{2+} elevation. *Comp. Biochem. Physiol.*, 116A: 11 – 16.

Sperelakis N, Bournaud R, 1991. Regulation of Ca^{2+} influx in myocardial cells by beta adrenergic receptors, cyclic nucleotides and phosphorylation. *Mol. Cell. Biochem.*, 82: 19 – 28.

Sumii K, Sperelakis N, 1995. cGMP-dependent protein kinase regulation of the L-type Ca^{2+} current in rat ventricular cyocytes. *Circ. Res.*, 77: 803 – 812.

(责任编辑: 黄玲巧)

cAMP 和 cGMP 对棉铃虫神经细胞高电压激活钙通道的调节作用

李 杰[1,2], 杜育哲[1], 佟 帆[1], 贺秉军[1], 刘安西[1]

(1.南开大学生命科学学院, 天津 300071; 2. 首都师范大学生物系, 北京 100037)

摘要: 用全细胞膜片钳法研究了 cAMP 和 cGMP 对棉铃虫 *Helicoverpa armigera* 3 龄幼虫胸腹神经节细胞高电压激活钙通道的调节作用。细胞外液中加入腺苷酸环化酶(AC)激活剂福斯克林(forskolin) 0.1 mmol/L, 对于 Ba^{2+} 介导的钙通道电流激活电压、峰电压、峰电流变化以及通道激活和电流达到峰值的时间无影响。电极内液中加入 1 mmol/L 的 cGMP 则明显抑制峰电流, 且抑制作用呈时间依赖性和浓度依赖性, 而对激活电压、峰电压无影响。结果提示, 棉铃虫神经细胞高电压激活钙通道的活动可能不受细胞内 cAMP 水平提高的影响, 但被 cGMP 抑制。

关键词: 棉铃虫; 环磷酸腺苷; 环磷酸鸟苷; 高电压激活钙通道; 膜片钳

中图分类号: Q965 **文献标识码**: A **文章编号**: 0454-6296(2004)04-0429-05